

# INTEGRATED EXTRACTION AND ANAEROBIC DIGESTION PROCESS FOR RECOVERY OF NUTRACEUTICALS AND BIOGAS FROM POMEGRANATE MARC

W. Qu, Z. Pan, R. Zhang, H. Ma, X. Chen, B. Zhu, Z. Wang, G. G. Atungulu

**ABSTRACT.** *Pomegranate marc (PM), a by-product of pomegranate juice processing, has not been effectively utilized. The objectives of this study were: (1) to determine the yields and properties of antioxidants (henceforth referring to total phenolics in terms of tannic acid equivalent) and oil extracted from various dry and wet constituents of PM, including peel, seeds, and mixture; and (2) to evaluate the anaerobic digestibility and biogas production potential of PM before and after antioxidant extraction (AE) and oil extraction (OE). Water and petroleum ether were used as solvents in the extraction of antioxidants and oil, respectively. The anaerobic digestion tests were conducted at  $35^{\circ}\text{C} \pm 2^{\circ}\text{C}$  with a feedstock to microorganism ratio of 0.5 on volatile solid (VS) basis under two initial organic loadings of 3.0 and 5.0 g VS  $\text{L}^{-1}$ . According to the results, both dry and wet PM extracts had similar extraction efficiency and functionality. The wet PM extract had an antioxidant content of 23.0%, which corresponded to an antioxidant yield of 106 kg per ton of PM peel on dry basis (d.b.). The DPPH scavenging activities of antioxidants were 6.5 to 6.6 g  $\text{g}^{-1}$  (d.b.). The oil yield from the dry PM seeds was 138 kg  $\text{ton}^{-1}$  (d.b.). Compared to the low initial organic loading, the high initial organic loading improved methane content (55.1% to 67.5%) but not biogas yield. The extracted residuals of peel, seeds, and mixture had methane yields of 148, 183, and 161 mL  $\text{g}^{-1}$  VS, respectively, which were lower than that from raw PM. Because the integrated process of extraction followed by anaerobic digestion can produce high functional antioxidants and high-quality biogas and oil from the PM, it is recommended as a value-added utilization method for the by-product.*

**Keywords.** *Anaerobic digestion, Antioxidant, Biogas, Methane, Pomegranate marc.*

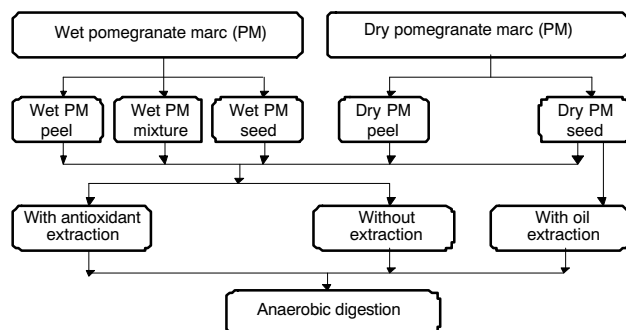
**P**omegranate (*Punica granatum* L.) is cultivated in California and Arizona for juice production in the U.S. In California alone, about 20.5 thousand tons of pomegranate fruits are produced each year, with 75% consumed fresh and 25% processed for juice production. The juice yield is approximately 322 to 341 L per ton of

fruit, which results in a large amount of pomegranate marc (PM) as a by-product, which is disposed of as waste or used as cattle feed. In the past, most studies have focused on antioxidants or oil extraction from pomegranate fruits and the properties of the extracts related to antioxidant, anticancer, and antimutagen, etc., (Abbasi et al., 2008; Adhami and Mukhtar, 2006; Heber et al., 2007; Kohno et al., 2004; Nigris et al., 2007; Yasoubi et al., 2007). The use of agricultural wastes as alternative low-cost sources of antioxidants (phenolics compounds) has been on the rise (Landbo and Meyer, 2001; Spigno and Faveri, 2007). Our recent research results showed that wet PM consists of approximately 77% peel and 21% seeds and is a good source of antioxidants and oil. However, the value-added utilization of extracted PM residues has not been studied. Therefore, development of integrated processing to recover nutraceuticals and energy from PM should bring economic and environmental benefits to pomegranate processors and producers (Qu et al., 2009).

Reported research (Singh et al., 2002) indicated that water, which is an environmentally friendly extraction method, can be an efficient extraction solvent for producing antioxidants from pomegranate peel and seeds. Preliminary research findings pointed out that antioxidants from pomegranate marc retained high quality at low extraction temperature and high water-to-sample ratio. The influential parameters for the extraction procedure included extraction solvent, temperature, and solid-to-liquid ratio (Bucic-Kojic et al., 2007; Lajpornik et al., 2005; Petersson et al., 2006).

Submitted for review in June 2009 as manuscript number FPE 8050; approved for publication by the Food & Process Engineering Institute Division of ASABE in October 2009.

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**Figure 1.** Process flowchart of the integrated system of antioxidant extraction, oil extraction, and anaerobic digestion.

Anaerobic digestion is a bioconversion technology that converts organic matter into biogas, which can be used as renewable fuel for heating or for co-generation of electricity and heat (Hobson and Wheatley, 1993). Due to the high moisture content of fruit-processing wastes, bio-conversion technologies, such as anaerobic digestion, are more suitable than thermo-conversion technologies, such as combustion and gasification (Bardiya et al., 1996). The biodegradability and biogas production potential of many types of fruit or vegetable wastes have been reported in the literature (Bouallagui et al., 2009; Bouallagui et al., 2005; Gomez et al., 2006; Viswanath et al., 1992). However, no report was found on biogas production from residues of pomegranate marc after antioxidant extraction (AE) and oil extraction (OE). It is important to determine the anaerobic digestibilities and biogas production potentials of AE and OE treated PM residues in order to develop an integrated system for producing value-added antioxidants and oil and biogas as renewable energy from pomegranate marc. Figure 1 shows a process flowchart of the new integrated system of antioxidant extraction, oil extraction, and anaerobic digestion. Because PM at present is regarded as a low-value product, a cost-effective approach is key if the extraction procedure is to be commercially viable. In our new approach to add value to PM marc, we took advantage of water as a viable and commercially representative extraction solvent.

The objectives of this study were: (1) to determine the yields and properties of antioxidants (total phenolics in terms of tannic acid equivalent) and oil extracted from various dry and wet constituents of PM, including peel, seeds, and mixture; (2) to examine the feasibility of converting the PM into biogas energy with different initial organic loadings before and after the extraction; and (3) to determine the kinetic parameters of biogas production using a modified Gompertz bacterial growth model.

## MATERIALS AND METHODS

### SAMPLE PREPARATION

The PM was obtained from a commercial pomegranate juice processor (Stiebs Pomegranate Products, Madera, Cal.) after the juice production processing of the “Wonderful” pomegranate variety. The PM was shipped to the USDA-ARS Western Regional Research Center in Albany, California, and stored at  $-18^{\circ}\text{C}$  until use.

In order to obtain dry PM, wet PM was dried using  $40^{\circ}\text{C}$  hot air in a cabinet drier (CPM Wolverine Proctor, LLC, Hor-

sham, Pa.) to lower the moisture content to 5% to 10%. The peel and seeds in the wet and dry forms, which were manually separated, and the mixture were ground three times using a grinder (Hobart N 50, Hobart Mfg. Co., Ltd., Troy, Ohio) equipped with a sieve of 5.0 mm opening. The ground samples were used for antioxidant extraction. Only dry seeds were used for oil extraction.

### ANALYSES OF PM SAMPLES AND MESOPHILIC INOCULUM

All PM samples before and after the extraction were analyzed for the properties related to biogas production. The pH value was determined at a distilled (DI) water to material ratio of 10:1  $\text{g g}^{-1}$ . The total carbon (TC), total nitrogen (TN), and carbon to nitrogen ratio (C/N) were measured based on the manufacturer’s procedure for the TruSpec CHN instrument (Leco, 2005). The moisture content, total solid (TS), and volatile solid (VS) were determined using standard methods (APHA, 1998) at the Bioenvironmental Engineering Research Laboratory of UC Davis. All reported weights are dry basis (d.b.) unless specified otherwise.

Mesophilic inoculum was collected from a working mesophilic anaerobic digester at a wastewater treatment plant in Davis, California. The inoculum was kept at  $4^{\circ}\text{C}$  before use. The inoculum was manually screened to remove the large solids using a screen with 1.8 mm opening. Prior to batch anaerobic digestion tests, the inoculum was incubated for three days at  $35^{\circ}\text{C} \pm 2^{\circ}\text{C}$  to allow stabilization. Its moisture content, VS, TS, and pH were determined. All analyses were performed in triplicate.

### ANTIOXIDANT EXTRACTION PROCEDURE

Water was used as the solvent for antioxidant extraction. The solid-liquid extraction was aided by the use of a magnetic stirring device (Isotemp, Fisher Scientific, Inc., Pittsburgh, Pa.). Based on the results of our preliminary study on antioxidant extraction (AE), the parameters for AE treatment were a DI water to sample ratio of 50:1  $\text{g g}^{-1}$ , extraction temperature of  $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ , reaction times of 4 h for wet and dry peel and 8 h for wet and dry seeds and mixture, and stirring speed of 1200 rpm. The liquid extract was separated from the residue by centrifugation (Marathon 21000R, Fisher Scientific, Inc., Pittsburgh, Pa.) at 3500 rpm for 20 min at  $4^{\circ}\text{C}$ . The liquid extract was analyzed to determine the extract yield, antioxidant yield, antioxidant content, and DPPH scavenging activity of antioxidants. All antioxidant extraction trials were carried out in triplicate.

### OIL EXTRACTION PROCEDURE

The oil in the dry seeds was extracted using petroleum ether (ACS grade, Fisher Scientific, Inc., Pittsburgh, Pa.) with ratio of petroleum ether to seed of 10:3  $\text{mL g}^{-1}$ , extraction temperature of  $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ , reaction time of 60 min, and stirring speed of 1200 rpm. The oil solution was filtered through Whatman No. 1 filter paper. The residue from the extraction was re-extracted using the above procedure. The oil solutions from the two extractions were combined and further dried to a constant weight by removing solvent in a rotary evaporator at  $45^{\circ}\text{C}$ . The yield and fatty acid composition of oil were determined according to the standard method (AOAC, 1995) by Anresco Laboratories (San Francisco, Cal.; www.anresco.com). The oil extraction (OE) tests were performed in triplicate.

## EXTRACTION EFFICIENCY AND PROPERTIES OF ANTIOXIDANTS AND OIL

The extract yield (%) was calculated based on the dry weight of extract and expressed as equation 1:

$$\text{Extract yield} = \frac{\text{g dried extract}}{100 \text{ g sample}} \times 100\% \quad (1)$$

The antioxidant content in the extract was determined by measuring the total phenolics, using a modified Folin-Ciocalteu method (Li et al., 2006). A volume of 60  $\mu\text{L}$  of extract was mixed thoroughly with 2 mL of sodium carbonate (7.5%) and 2.5 mL of Folin-Ciocalteu reagent (10-fold dilute by DI water) using a vortex mixer. The mixed solution was held in water bath for 30 min at  $25^\circ\text{C} \pm 2^\circ\text{C}$ , and then its absorbance was measured at 760 nm using a spectrophotometer (Genesys 10Bio, Thermo Fisher Scientific, Inc., Waltham, Mass.). The blank was prepared as above, but the extract was replaced by the same volume of DI water. The antioxidant was expressed as tannic acid equivalent (TAE) using a tannic acid (0 to 0.014  $\text{g L}^{-1}$ ) standard curve. The antioxidant yield (%) and antioxidant content (%) were calculated using equations 2 and 3:

$$\text{Antioxidant yield} = \frac{\text{g total phenolics}}{100 \text{ g sample}} \times 100\% \quad (2)$$

$$\text{Antioxidant content} = \frac{\text{g total phenolics}}{100 \text{ g dried extract}} \times 100\% \quad (3)$$

The antioxidant activity was estimated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method based on DPPH reduction gram per gram of antioxidants using an adapted colorimetric procedure (Singh et al., 2002). A volume of 60  $\mu\text{L}$  of extract was added to 3 mL of DPPH solution in methanol (0.05  $\text{g L}^{-1}$ ). The sample solution was mixed thoroughly using a vortex mixer and held in water bath for 20 min at  $25^\circ\text{C} \pm 2^\circ\text{C}$ . The sample absorbance was measured at 517 nm using a spectrophotometer. The control solution included 60  $\mu\text{L}$  of DI water and 3 mL of DPPH solution in methanol (0.05  $\text{g L}^{-1}$ ). The blank solution contained 60  $\mu\text{L}$  of extract and 3 mL of methanol. The DPPH scavenging activity,  $DSA$  ( $\text{g g}^{-1}$ ), was calculated using equation 4:

$$DSA = \frac{[C_u - (C_v - C_w) \times n_0 V]}{W_0} \quad (4)$$

where

- $DSA$  = DPPH scavenging activity ( $\text{g g}^{-1}$ )
- $C_u$  = DPPH concentration in the control solution ( $\text{g L}^{-1}$ )
- $C_v$  = DPPH concentration in the sample solution ( $\text{g L}^{-1}$ )
- $C_w$  = DPPH concentration in the blank solution ( $\text{g L}^{-1}$ )
- $n_0$  = dilution multiple of extract
- $V$  = total volume of liquid extract (L)
- $W_0$  = dry weight of antioxidant (g).

The oil yield (%) was calculated based on the dry weight of oil using equation 5:

$$\text{Oil yield} = \frac{\text{g dried oil}}{100 \text{ g sample}} \times 100\% \quad (5)$$

## EXPERIMENTAL DESIGN OF BATCH ANAEROBIC DIGESTION

The biodegradabilities of PM samples before and after extraction were determined using batch anaerobic digestion tests, as described in Anaerobic Lab Work (2000). The conditions for batch anaerobic digestion are as follows: digestion temperature of  $35^\circ\text{C} \pm 2^\circ\text{C}$ , feedstock to microorganism ratio (F/M) of 0.5 on VS basis, initial organic loadings of 3.0 and 5.0  $\text{g VS L}^{-1}$ , and working volume of 0.5 L in 1 L glass bottles (Kimax No. 14397). All trials were conducted in triplicate.

In order to achieve the initial organic loading of 5.0  $\text{g VS L}^{-1}$ , the reactors were loaded with 2.5 g VS of feedstock, 5 g VS of inoculum, and water added to a final total volume of 0.5 L. To achieve the loading of 3.0  $\text{g VS L}^{-1}$ , the reactors were loaded with 1.5 g VS of feedstock, 3 g VS of inoculum, and water added to a final total volume of 0.5 L. The reactors were tightly closed with rubber septa and screw caps. Prior to the start of each digestion test, the headspace of each reactor was purged with argon gas for 5 min to ensure the anaerobic condition. Then all reactors were incubated at  $35^\circ\text{C} \pm 2^\circ\text{C}$  for a period of time until the daily biogas production from the reactors became negligible. Three blank reactors contained the same amount of inoculum and were filled up to 0.5 L with tap water. They were used to measure the biogas production from the inoculum. The biogas yield from the substrate was calculated by subtracting the biogas yield of the blank reactors from the biogas production of the testing reactors.

## MEASUREMENTS AND CALCULATIONS OF BIODEGRADABILITY AND BIOGAS PRODUCTION POTENTIAL

Biogas production was determined daily by measuring the pressure in the headspace of each reactor and then converting it to volume by application of the ideal gas law. Pressure was measured using a membrane pressure gauge (type 3150, WAL Mess- und Regelsysteme GmbH, Oldenburg, Germany). After the pressure measurement, the biogas in the headspace was released under water. Then the pressure in the headspace was measured again as an initial condition for the next-day measurement. Daily pressure differences were converted into biogas volume. The volume of daily biogas production,  $V_t$  (mL), was calculated using equation 6:

$$V_t = \frac{(P_t - P_{t-1}) \cdot T_a}{P_a \cdot T_r} \cdot V_h \quad (6)$$

where

- $V_t$  = volume of daily biogas production in day  $t$  (mL)
- $P_t$  = absolute headspace pressure before release in day  $t$  (kPa)
- $P_{t-1}$  = absolute headspace pressure after release in previous day  $t-1$  (kPa)
- $V_h$  = headspace volume of the reactor (mL)
- $P_a$  = ambient pressure (kPa)
- $T_a$  = ambient temperature (K)
- $T_r$  = absolute temperature of the reactor (K).

Biogas production rate ( $\text{mL L}^{-1} \text{d}^{-1}$ ) was determined by dividing the volume of daily biogas production by the reactor working volume (0.5 L). Cumulative biogas yield ( $\text{mL g}^{-1} \text{VS}$ ) was calculated based on the total volume of biogas produced during the entire test period per gram of initial VS contained in the substrate. The VS reduction (%) was

estimated as the percentage loss of VS in the substrate at the end of the test.

The methane content of biogas was measured daily using a gas chromatography (GC) (GC6890N, Agilent Technologies, Inc., Santa Clara, Cal.) equipped with a thermal conductivity detector (TCD) and packed column (C-9000, Alltech Associates, Inc., Deerfield, Ill.) of 3.05 m length, 3.18 mm outside diameter, 2.16 mm internal diameter, and with 80/100 mesh carbosphere. Argon was the carrier gas at a flow rate of 30 mL min<sup>-1</sup>. The temperatures of the injector, oven, and detector were 120°C, 100°C, and 120°C, respectively. A standard gas (Scott Specialty Gases, Inc., Plumsteadville, Pa.) with 60% (v/v) CH<sub>4</sub> and 40% (v/v) CO<sub>2</sub> was used to calibrate the GC. Each GC analysis was performed in triplicate. The methane yield, *MY* (mL g<sup>-1</sup> VS), was calculated using equation 7:

$$MY = \frac{\sum_{x=1}^n (V_x \times MC_x)}{W_1} \quad (7)$$

where

- MY* = methane yield (mL g<sup>-1</sup> VS)
- V<sub>x</sub>* = volume of daily biogas production on day *x* (mL)
- MC<sub>x</sub>* = methane content on day *x* (%)
- W<sub>1</sub>* = VS contained in the substrate (g)
- n* = total number of observations (day).

#### SIMULATION OF BATCH ANAEROBIC DIGESTION

A modified model of Gompertz bacterial growth (Schnute, 1981) was used to calculate kinetics parameters of biogas production for predicting the biogas yield potential (eq. 8):

$$CBY = BYP \exp \left\{ - \exp \left[ 1 - \frac{BPR_M \times V_h K}{BYP \times 1000 W_1} (t - \lambda) \right] \right\} \quad (8)$$

where

- CBY* = cumulative biogas yield (mL g<sup>-1</sup> VS)
- BYP* = biogas yield potential (mL g<sup>-1</sup> VS)
- BPR<sub>M</sub>* = maximum biogas production rate (mL L<sup>-1</sup> d<sup>-1</sup>)
- λ* = lag time of bacteria growth (d)
- t* = digestion time (d)
- K* = mathematical constant (2.718)
- V<sub>h</sub>* = headspace volume of the reactor (mL)
- W<sub>1</sub>* = VS contained in the substrate (g VS).

The values of *BYP*, *BPR<sub>M</sub>*, and *λ* were nonlinearly determined using single Levenberg-Marquardt iteration with OriginPro 7.5 SR1 (v. 7.5776, OriginLab Corp., Northampton, Mass.).

#### STATISTICAL ANALYSES

Data analysis was performed using SAS (v. 9.2, SAS Institute, Inc., Cary, N.C.) to determine the levels of significant differences in the extract yield, antioxidant yield, antioxidant content, and DPPH scavenging activity of different sample types, and biogas yield and methane content of biogas under the different initial loadings, as well as the biogas production potential of untreated and treated PM samples. The significance tests were based on Tukey's Studentized range test and least significant difference (LSD) ( $\alpha = 0.05$ ).

## RESULTS AND DISCUSSION

### EXTRACTION EFFICIENCY AND PROPERTIES OF ANTIOXIDANTS AND OIL

Table 1 shows the extract yields, antioxidant yields, antioxidant contents, and DPPH scavenging activities of PM samples. The drying process showed no significant effect on the extraction efficiency and functionality of the extract from either peel or seeds. Therefore, direct usage of the PM in either wet or dried form for antioxidant (total phenolics in terms of tannic acid equivalent) production is feasible. The antioxidants extracted from all samples had no significant difference in DPPH scavenging activities (6.1 to 6.9 g g<sup>-1</sup>). The peel had significantly high extract yields, antioxidant yields, and antioxidant contents compared to the seeds and mixture. We speculated that was mainly due to their inherent physical and chemical differences. The peel contains much less cellulosic compounds and has a looser physical structure than the seeds, which could allow better extraction efficiency. Therefore, the peel of pomegranate marc could be better source for producing antioxidant than the seeds and mixture. For instance, whereas one ton wet PM peel could produce 106 kg of antioxidant with DPPH scavenging activity of 6.6 g g<sup>-1</sup>, only 41 kg of antioxidant with DPPH scavenging activity of 6.9 g g<sup>-1</sup> could be produced from one ton of wet PM mixture.

The antioxidant (total phenolics) yields and contents obtained in this research are much higher than that reported by Singh et al. (2002). They observed the same phenolics yields of 7.73% with contents of 3% from both pomegranate peel and seeds. But the DPPH scavenging activity of phenolics from the peel was 70%, which was significantly higher than that from the seeds (30%) at phenolics concentration of 50 ppm. The differences might be due to differences in varieties of pomegranate and compositions of the pomegranate marc used.

Based on the measured result of oil yield, one ton of dry PM seeds produced 138 kg of oil. The fatty acid composition of PM seed oil is represented in table 2. The oil was rich in unsaturated fatty acids of linoleic (33%), docosahexaenic (0.37%), and linolenic acids (0.1%). The oil also contained many saturated fatty acids of palmitic acid (14.9%) and stearic acid (11%). Similar reports noted that pomegranate seed oil was rich in C16 and C18 fatty acids (Abbasi et al., 2008; Fadavi et al., 2006). The PM seed oil contained more palmitic, stearic, palmitoleic, and oleic acids than the grape seed oil reported by Gomez et al. (1996). Therefore, the seeds of pomegranate marc are good sources for producing oil with high unsaturated fatty acids.

**Table 1. Yields and properties of extracts from wet and dry PM samples using water as solvent.<sup>[a]</sup>**

Sample Type	Extract Yield (%)	Antioxidant		DPPH Scavenging Activity (g g <sup>-1</sup> )
		Yield (%)	Content (%)	
Wet PM peel	46.3 a	10.6 a	23.0 a	6.6 a
Dry PM peel	50.8 a	10.2 a	20.1 a	6.5 a
Wet PM seed	22.1 c	1.1 c	5.2 c	6.1 a
Dry PM seed	28.6 bc	1.4 c	5.1 c	6.8 a
Wet PM mixture	29.8 b	4.1 b	13.8 b	6.9 a

<sup>[a]</sup> Different letters indicate significant difference at  $P < 0.05$ . Yield is the dry basis of the raw material, and DPPH scavenging activity is the amount of DPPH oxidized based on antioxidant weight.

**Table 2. Fatty acid composition of extracted oil from dry PM seeds.**

Fatty Acid	% d.b.
Myristic (C14:0)	0.10
Palmitic (C16:0)	14.90
Margaric (C17:0)	0.25
Stearic (C18:0)	11.00
Arachidic (C20:0)	2.62
Heneicosanoic (C21:0)	0.26
Behenic (C22:0)	0.57
Tricosanoic (C23:0)	0.52
cis-10 Pentadecenoic (C15:1)	0.33
Palmitoleic (C16:1)	2.45
cis-10 Heptadecenoic (C17:1)	0.29
Oleic (C18:1)	28.80
Linoleic (C18:2)	33.00
Linolenic (C18:3)	0.10
Gondoic (C20:1)	3.44
cis 11, 14 Eicosadienoic (C20:2)	0.13
cis 13, 16 Docosadienoic (C22:2)	0.16
Nervonic (C24:1)	0.78
cis 4, 7, 13, 16, 19 Docosahexaenic	0.37

### CHARACTERISTICS OF PM SAMPLES AND MESOPHILIC INOCULUM

Table 3 lists the TC, TN, C/N, moisture content, VS/TS, and pH of PM samples and mesophilic inoculum. The results showed that the wet and dry samples had similar TC, TN, C/N, VS/TS, and pH, which indicated that the drying process did not change the chemical characteristics. The seeds had significantly higher TN (1.9% to 2.7% d.b.) and lower C/N (19.3 to 26.1) than the peel (TN of 0.8% to 1.3% and C/N of 38.3 to 58.5). Because antioxidants are mainly made up of carbon element (Gil et al., 2000), the C/N values of AE treated samples were decreased by 11.1% to 34.4% compared to that of untreated samples. The C/N value of OE treated sample was reduced by 12.3% due to the loss of fatty acids with much carbon element. However, the VS/TS values were not affected by the AE and OE treatments. The extracted PM residues still contained high VS/TS (96.9% to 99.1%) like some fruit or vegetable wastes, such as banana peels (87% to 95%), jackfruit peels (83% to 92%), apple pomace (96% to 98%), and sugar beet pulp (90% to 95%) (Bardiya et al., 1996; Hutnan et al., 2000; Krishnan, et al., 2006; William and Robert, 1984). In view of the high VS/TS and C/N, the PM residues from antioxidant extraction and oil extraction were still suitable substrates for anaerobic digestion.

**Table 3. Characteristics of untreated and treated PM samples and mesophilic inoculum.<sup>[a]</sup>**

Treatment	Total C (% d.b.)	Total N (% d.b.)	C/N	MC (%)	VS/TS (%)	Material pH
Wet peel						
Untreated	47.4	0.8	56.5	74.3	95.8	3.4
AE treated	48.4	1.3	38.3	89.5	98.3	4.2
Dry peel						
Untreated	46.9	0.8	58.5	12.7	95.8	3.4
AE treated	48.2	1.3	38.4	83.8	97.9	3.9
Wet seed						
Untreated	50.2	2.3	21.7	64.3	97.0	3.6
AE treated	51.4	2.7	19.3	81.4	99.1	4.4
Dry seed						
Untreated	49.4	1.9	26.1	6.9	97.1	3.9
AE treated	51.7	2.5	20.9	76.5	98.5	3.8
OE treated	47.4	2.1	22.9	8.7	96.9	4.1
Wet mixture						
Untreated	47.7	1.3	36.0	77.2	96.2	3.4
AE treated	49.5	1.9	25.7	87.9	98.6	4.1
Mesophilic inoculum	N/A	N/A	N/A	97.7	55.0	7.4

[a] MC = moisture content, d.b. = dry basis, and N/A= not available.

The moisture content, VS/TS, and pH of the mesophilic inoculum were 97.7%, 55.0%, and 7.4, respectively. Even though pH values (3.4 to 4.4) of PM samples were acidic, the initial pH values of the solutions in the reactors were near neutral (7.1 to 7.3) after adding inoculum at an F/M ratio of 0.5. The neutral pH was a satisfactory environment for anaerobic digestion (Verrier et al., 1987; Zoetemeyer et al., 1982).

### EFFECT OF INITIAL ORGANIC LOADING ON BIODEGRADABILITY OF EXTRACTED PM RESIDUES

Table 4 shows the biogas yields, methane contents of biogas, VS reductions, initial pH, and final pH of AE and OE treated PM samples at two initial loadings of 3.0 and 5.0 g VS L<sup>-1</sup>. Statistical analyses indicated that biogas produced from extracted samples at the two loadings gave similar yields. However, significantly high methane contents of biogas were observed at the high initial loading compared to that at the low initial loading. The VS reductions at the low and high initial loadings were very similar for the same sample. Reported results mentioned that the methane production

**Table 4. Biogas yields, methane contents of biogas, VS reductions, initial pH, and final pH of the extracted residues at two initial loadings.<sup>[a]</sup>**

Sample Type	Initial Loading (g VS L <sup>-1</sup> )	Biogas Yield (mL g <sup>-1</sup> VS)	Methane Content of Biogas (%)	VS Reduction (%)	Initial pH	Final pH
AE treated wet peel	3.0	286 a	44.5 b	12.2	7.1	6.9
	5.0	276 a	56.7 a	11.6	7.1	6.8
AE treated dry peel	3.0	274 a	46.1 b	14.4	7.1	6.9
	5.0	267 a	55.1 a	13.1	7.2	6.8
AE treated wet seed	3.0	305 a	52.1 b	20.4	7.3	6.9
	5.0	298 a	65.5 a	19.1	7.1	6.9
AE treated dry seed	3.0	360 a	55.7 b	20.4	7.3	7.0
	5.0	346 a	67.5 a	18.5	7.2	6.8
OE treated dry seed	3.0	378 a	57.8 b	21.4	7.1	6.9
	5.0	353 a	63.0 a	20.5	7.2	6.9

[a] Different letters indicate significant difference between initial loadings of 3.0 and 5.0 g VS L<sup>-1</sup> within the same type at P < 0.05.

increased with increased initial loading during the anaerobic digestion (Mata-Alvarez et al., 1992). The initial and final pH values in the reactors were similar and kept at neutral during the batch anaerobic digestion. This indicated that little volatile fatty acid (VFA) was accumulated, and the digesters ran steadily during the batch anaerobic digestion because the unsuitable pH (<6.5 or >8.0) tended to decrease the rate of methanogenesis (Verrier et al., 1987; Zoetemeyer et al., 1982).

#### EFFECTS OF EXTRACTION ON BIOGAS YIELD AND BIOGAS PRODUCTION POTENTIAL

Because the high initial loading resulted in significantly higher methane contents than the low initial loading, only the results of anaerobic digestion and biogas production potential at the high initial loading of 5.0 g VS L<sup>-1</sup> are reported below.

During batch anaerobic digestion of untreated and AE treated wet PM samples (fig. 2), the cumulative biogas productions increased rapidly during the first 3 days and then slowed down. More than 95% of the biogas production from untreated wet peel, seeds, and mixture was achieved within 8, 14, and 14 days, respectively. The AE treated wet peel, seeds, and mixture were digested more slowly and required 13, 16, and 16 days, respectively, to attain 95% of the biogas yields. The untreated wet peel, seeds, and mixture showed higher biogas yields (396, 405, and 389 mL g<sup>-1</sup> VS, respectively) than the AE treated wet samples, which had corresponding biogas yields of 276, 298, and 292 mL g<sup>-1</sup> VS, respectively, at a digestion time of 20 days. The maximum peak values of the biogas production rate from the untreated wet peel, seeds, and mixture were reached on the first day, with values of 676, 525, and 611 mL L<sup>-1</sup> d<sup>-1</sup>, respectively, compared to corresponding values of 365, 197, and 293 mL L<sup>-1</sup> d<sup>-1</sup>, from the AE treated samples, which were reached on the second day. Thus, the AE process significantly reduced the cumulative biogas yields and biogas production rates, but increased the digestion time. This is because the extract removal reduced the utilizable soluble substrate for the inoculum, which resulted in reduced bacterial growth and biogas production.

Both untreated and AE treated wet seeds had lower biogas production rates than wet peel, and their second peak of

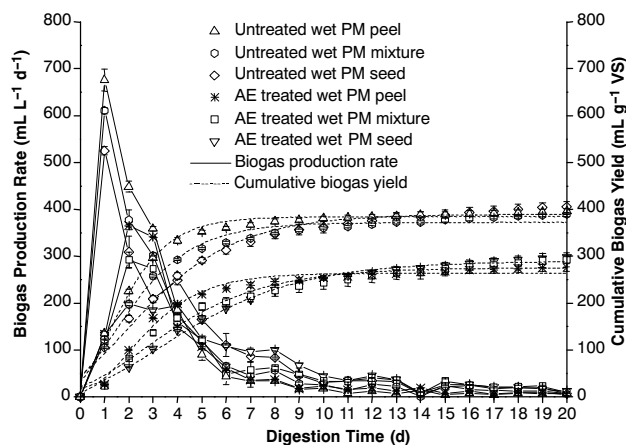


Figure 2. Cumulative biogas yields and biogas production rates of untreated and AE treated wet PM samples during batch anaerobic digestion at 35°C ± 2°C and initial organic loading of 5.0 g VS L<sup>-1</sup>.

biogas production rate appeared on the fourth day of digestion. This was likely because the seeds had more hardly degradable substances, such as lignin, cellulose, and hemicellulose compared to the peel. These cellulosic compounds need longer digestion time to be digested. The peel, with a loose and soft structure, exposed more interaction area with the inoculum and was easily digestible.

The cumulative biogas yields and biogas production rates of untreated, AE treated, and OE treated dry PM samples are shown in figure 3. The curve trends of cumulative biogas yields and biogas production rates from dry peel and seeds, either untreated or AE treated, were similar to those from the wet samples but had slightly higher values, which could be due to the different particle sizes of the wet and dry samples from the milling operation. At anaerobic digestion time of 20 days, the untreated dry peel and seeds had higher biogas yields (418 and 455 mL g<sup>-1</sup> VS, respectively) compared to the AE treated samples, which had biogas yields of 267 and 346 mL g<sup>-1</sup> VS, respectively. The corresponding first peak values of the biogas production rates for untreated dry peel and seeds were 693 and 591 mL L<sup>-1</sup> d<sup>-1</sup>, respectively, whereas the AE treated samples had rates of 333 and 297 mL L<sup>-1</sup> d<sup>-1</sup>.

For the seed residue from OE, the curve trends of cumulative biogas yield and biogas production rate were different from those for AE treated seed residues, but were in agreement with those for the untreated seeds. This indicated that oil extraction did not affect the water-soluble substrate that was used for biogas production. However, both the biogas yield and biogas production rate were reduced by the oil extraction process. The seed residue from OE reached a cumulative biogas yield of 353 mL g<sup>-1</sup> VS at an anaerobic digestion time of 20 days with a maximum rate of 569 mL L<sup>-1</sup> d<sup>-1</sup>.

Table 5 shows the biogas yield potential (*BYP*), maximum biogas production rate (*BPR<sub>M</sub>*), and bacteria growth lag time (*λ*) of untreated and treated PM samples, which were obtained using the modified Gompertz kinetics model (eq. 8). The model fitted the experimental data very well, with coefficients of determination (*R*<sup>2</sup>) of 0.957 to 0.994. The results also showed that the bacteria growth lag time was delayed by antioxidant extraction compared to oil extraction. This is because oil extraction did not remove the water-soluble substrate required for the inoculum to produce

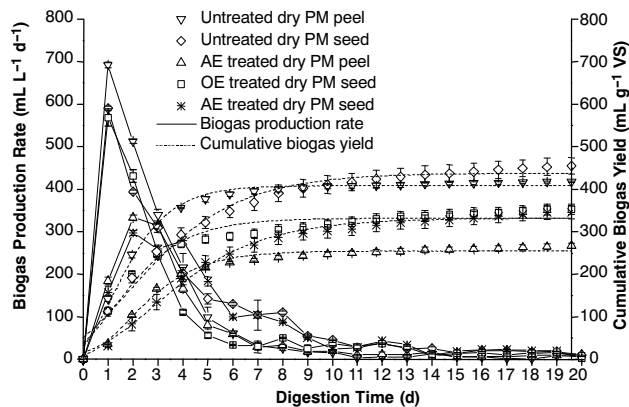
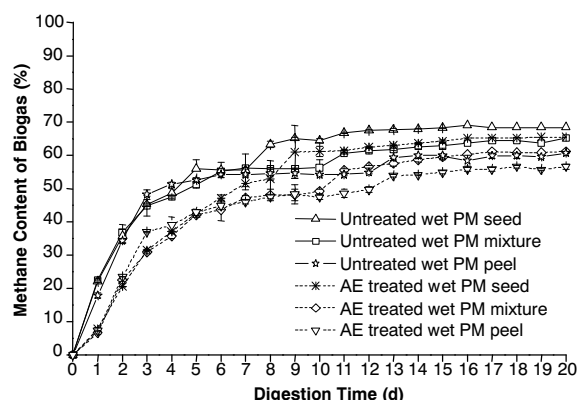


Figure 3. Cumulative biogas yields and biogas production rates of untreated, AE treated, and OE treated dry PM samples during batch anaerobic digestion at 35°C ± 2°C and initial organic loading of 5.0 g VS L<sup>-1</sup>.

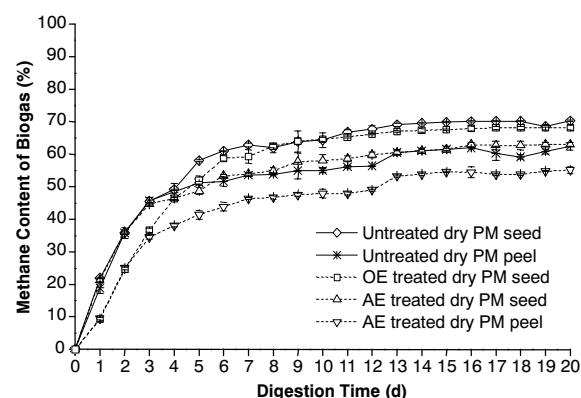
**Table 5. Calculated biogas yield potential, maximum biogas production rate, and bacteria growth lag time of untreated and treated PM samples using the modified Gompertz model.**

Sample Type	Treatment	Biogas Yield Potential <sup>[a]</sup> (mL g <sup>-1</sup> VS)	Maximum Biogas Production Rate		Bacteria Growth Lag Time (d)	R <sup>2</sup>
			(mL g VS <sup>-1</sup> d <sup>-1</sup> )	(mL L <sup>-1</sup> d <sup>-1</sup> )		
Wet PM peel	Untreated	384 a	107	536	0	0.988
	AE treated	264 b	59	296	0.4	0.987
Dry PM peel	Untreated	409 a	117	583	0	0.991
	AE treated	255 b	60	298	0.1	0.980
Wet PM seed	Untreated	390 a	57	284	0	0.984
	AE treated	290 b	33	166	0.1	0.994
Dry PM seed	Untreated	438 a	64	322	0	0.982
	AE treated	332 b	48	242	0.3	0.992
	OE treated	332 b	55	277	0	0.957
Wet PM mixture	Untreated	372 a	77	387	0	0.978
	AE treated	276 b	37	183	0.1	0.975

<sup>[a]</sup> Different letters indicate significant difference between untreated and treated within the same type at P < 0.05.



**Figure 4. Methane contents of biogas from untreated and AE treated wet PM samples during batch anaerobic digestion at 35 °C ± 2 °C and initial organic loading of 5.0 g VS L<sup>-1</sup>.**



**Figure 5. Methane contents of biogas from untreated, AE treated and OE treated dry PM samples during batch anaerobic digestion at 35 °C ± 2 °C and initial organic loading of 5.0 g VS L<sup>-1</sup>.**

biogas, in contrast to antioxidant extraction. The AE and OE treated samples had significantly lower biogas yield potential and maximum biogas production rates than the untreated samples.

#### EFFECTS OF EXTRACTION ON METHANE CONTENT OF BIOGAS AND METHANE YIELD

The methane contents of biogas from untreated and treated PM samples in wet and dry forms are shown in figures 4 and 5. The wet and dry samples had similar trend in their curves. The methane contents of biogas increased sharply during the first 3 days and then slowed down to stable values. The untreated wet peel, seeds, and mixture gave higher methane contents (60.8%, 68.5%, and 65.2%, respectively) than the AE treated samples, which had methane contents of 56.7%, 65.5%, and 61.2%, respectively, at digestion time of 20 days. The biogas from untreated dry peel and seeds had even high methane contents of 62.5% and 70.3% at digestion time of 20 days, compared to 55.1% and 67.5% from AE treated samples and 63.0% from OE treated dry seeds.

Table 6 shows the methane yields from untreated and treated PM samples at digestion time of 20 days. The effects of drying and extraction on methane yields were similar to the effects on biogas production. The extraction process caused

**Table 6. Methane yields and VS reductions of untreated and treated PM samples using batch anaerobic digestion at digestion time of 20 days.**

Sample Type	Treatment Condition	Methane Yield (mL g <sup>-1</sup> VS)	VS Reduction (%)
Wet PM peel	Untreated	207	16.4
	AE treated	148	11.6
Dry PM peel	Untreated	213	17.6
	AE treated	140	13.1
Wet PM seed	Untreated	249	19.6
	AE treated	183	19.1
Dry PM seed	Untreated	276	22.7
	AE treated	222	18.5
	OE treated	200	20.5
Wet PM mixture	Untreated	221	16.3
	AE treated	161	13.2

the loss of biodegradable substrate for methanogenesis, which correspondingly reduced the methane yield.

#### EFFECT OF EXTRACTION ON VOLATILE SOLID REDUCTION

The VS reductions of extracted PM peel, seeds, and mixture in wet and dry forms were 11.6% to 13.1%, 18.5% to 20.5%, and 13.2%, respectively, which were lower than those (16.4% to 17.6%, 19.6% to 22.7%, and 16.3%) of the untreated samples. The VS reduction had a positive

**Table 7. Products and related properties from one ton of dry mass pomegranate marc using different processing methods.<sup>[a]</sup>**

Raw Material	Fraction	Percentage (% d.b.)	Extraction Treatment	Products and Related Properties				
				Oil (kg d.w.)	Extract (kg d.w.)	Antioxidant Content (% d.b.)	Biogas (m <sup>3</sup> )	Methane Content (%)
Wet	Mixture	100	No	--	--	--	376	65.2
			Yes	--	298	13.8	204	61.2
Dry	Peel	64	No	--	--	--	257	62.5
	Seeds	36	No	--	--	--	159	70.3
	Total	100	No	--	--	--	416	65.6
	Peel	64	Yes	--	325	20.1	83	55.1
	Seeds	36	Yes	50	--	--	106	63.0
	Total	100	Yes	50	325	20.1	189	59.8

<sup>[a]</sup> d.b. = dry basis; d.w. = dry weight.

relationship with biogas and methane production (Bardiya et al. 1996).

#### VOLATILE SOLID AND DIGESTION ACTIVITIES

Generally, digester performance is highly sensitive to the quality of the feed; the yield and kinetics of the biological reaction involved in anaerobic digestion are strongly dependent upon the waste composition (Archana et al., 1999). The easy-operation batch digestion design that uses highly active anaerobic inoculum and mesophilic conditions, as adapted in our study, is often used in related basic research. We speculate that changing the reactor design would slightly affect the process (William and David, 1999). In recent years, a number of novel reactor designs have been adapted and developed, allowing a significantly higher rate of reaction per unit volume of reactor. We observed that the VS reductions and digestion activities of pomegranate samples after 7 days were low, as shown in tables 4 and 6 and figures 2 and 3. Although this phenomenon could be related to factors such as microorganism characteristics, digestion environment, and reactor design, it has no impact on the trend of results of control and treatment in the experimental design. Based on the characteristics of pomegranate marc samples analyzed in table 3, relatively good C/N and VS/TS ratios were observed, and these findings clearly indicated that the pomegranate marc could produce biogas and had utilizable nutritional substance. It is therefore speculated that the low VS reductions of the treated samples were primarily because the pomegranate residues, after antioxidant or oil extraction, contained less utilizable substrate for the inoculum. The low digestion activity could have occurred because the leftover pomegranate marc, after 7 days of digestion, consisted of hardly degradable substances, such as lignin, cellulose, and hemicellulose, which could cause low daily biogas production and biogas yield, especially for the extracted residues. Clarifying the compositional changes during biodegradation of the substrate is therefore a subject for further study. However, it is clear that the extraction process significantly decreased the biogas production potential, and regardless of the low VS reduction and digestion activity, the pomegranate marc still had good potential for biogas production and methane content (tables 5 and 6).

#### PERFORMANCE OF INTEGRATED PROCESS

Table 7 shows the products and related properties from one ton of dry mass of PM when different processing methods were used. When the wet PM mixture was directly used to

produce biogas, it produced 376 m<sup>3</sup> of biogas with methane content of 65.2%. In contrast, when the mixture was processed using the integrated process of extraction followed by digestion, the products included 298 kg of extract with antioxidant content of 13.8%, and 204 m<sup>3</sup> of biogas with methane content of 61.2%. Similarly, when the PM was dried and then directly digested, it produced 416 m<sup>3</sup> of biogas with methane content of 65.6%. When the dry mixture was separated to obtain peel and seeds, it produced 640 kg of peel and 36 kg of seeds. If the dry seeds and peel are used to produce oil and antioxidants, and the extracted residues of the seeds and peel are then digested for producing biogas, the products will include 50 kg of high-quality oil, 325 kg of extract with antioxidant content of 20.1%, and 189 m<sup>3</sup> of biogas with methane content of 59.8%. Obviously, because the integrated process can produce a large amount of antioxidants, high-quality oil, and significant amount of biogas, it should be an economically viable approach to value-added utilization of pomegranate marc.

#### CONCLUSIONS

The drying process did not show any significant effect on the extraction efficiency and functionality of the extract from either PM peel or PM seeds. This indicated that the by-product from pomegranate juice processing can be directly used for antioxidant production (total phenolics in terms of tannic acid equivalent) or dried first when necessary. One ton of dry mass from wet PM peel can produce 508 kg of extract. The extract had antioxidant content of 23.0%, which corresponded to an antioxidant yield of 106 kg per ton of PM peel on dry basis (d.b.). The PM seeds and mixture produced much less antioxidants than the peel. The antioxidants from the PM peel, seeds, and mixture had similar DPPH scavenging activities (6.1 to 6.9 g g<sup>-1</sup>). One ton of dry mass from dry PM seeds can produce 138 kg of oil with high unsaturated fatty acids.

Compared to the low initial loading (3.0 g VS L<sup>-1</sup>), the high initial loading (5.0 g VS L<sup>-1</sup>) improved methane contents (55.1% to 67.5%) but not biogas yield. With initial loading of 5.0 g VS L<sup>-1</sup>, the extracted residuals of peel, seeds, and mixture had low biogas yields of 276, 298, and 292 mL g<sup>-1</sup> VS, respectively, and methane yields of 148, 183, and 161 mL g<sup>-1</sup> VS, compared to the raw PM samples with biogas yields of 396, 405, and 389 mL g<sup>-1</sup> VS and methane yields of 207, 249, and 221 mL g<sup>-1</sup> VS, at digestion time of 20 days. The modified Gompertz model was adequate for predicting

the biogas production potential. Because high functional antioxidants and high-quality biogas and oil can be produced by sequential extractions and anaerobic digestion, the integrated process should be an economically viable approach to utilizing pomegranate marc.

## ACKNOWLEDGEMENTS

This research was conducted at the USDA-ARS Western Regional Research Center and the University of California, Davis. The authors wish to thank Donald Olson and Kameron Chun for their technical support in pomegranate drying and GC training. The authors also wish to extend thanks to Stiebs Pomegranate Products, Madera, California, for providing the PM materials.

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